510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K043067

B. Purpose for Submission:
New Device

C. Measurand:
Anti-ENA antibodies (SSA, SSB, Sm, Sm/RNP, Scl-70, and Jo-1)

D. Type of Test:
Microarray based immunoassay, Qualitative

E. Applicant:
BioArray Solutions, Ltd.

F. Proprietary and Established Names:
ENA IgG BeadChip™ Test System on the Array Imaging System (AIS 400)

G. Regulatory Information:
1. Regulation section:
   21 CFR 866.5100, Antinuclear antibody immunological test system
2. Classification:
   II
3. Product code:
   LLL, Extractable antinuclear antibody, antigen and control
4. Panel:
   Immunology 82

H. Intended Use:
1. Intended use(s):
   The BioArray Solutions ENA IgG BeadChip Test System is intended for use in testing human serum for the presence of human IgG class antibodies to six extractable nuclear antigens: SSA, SSB, Sm, Sm/RNP, Scl-70, and Jo-1. The presence of these autoantibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of systemic lupus erythematosus, Sjögren's syndrome, scleroderma and myositis. This ENA IgG BeadChip is for use with the BioArray Solutions Array Imaging System (AIS 400). This test is for in vitro diagnostic use.
2. Indication(s) for use:
   Same as Intended use
3. Special conditions for use statement(s):
   For Prescription use
4. Special instrument requirements:
   Array Imaging System (AIS 400)

I. Device Description:

The ENA IgG BeadChip™ Test System consists of an ENA IgG BeadChip (12 carriers x 8-BeadChip arrays), a positive human serum control, a negative human serum control, sample diluent/wash buffer and goat anti-human detection conjugate.

The AIS 400 system is an automated image acquisition system with an integrated analysis system designed for BioArray Solutions BeadChip™ microarrays. The major components are the microscope, automatic stage, focus motor, light source, CCD camera, computer and a software package that includes BioArray Solution Reader (BASR), Client Merger, and New Batch programs.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ENA IgG ImmuStrip™ Test System, Distributed by SLR Research Corporation
2. Predicate 510(k) number(s):
   K964787
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	Intended for use in testing human serum for the presence of human IgG class antibodies to six extractable nuclear antigens to aid in the diagnosis of systemic lupus erythematosus, Sjögren's syndrome, scleroderma and myositis.	Same
Assay Type	Immunoassay	Same
Antigens	SSA, SSB, Sm, Sm/RNP, Scl-70, and Jo-1	Same
Assay format	Qualitative	Same
Sample Type	Serum	Same

Differences		
Item	Device	Predicate
Technology	Microarray based immunoassay	ELISA
Solid phase	Microparticle	Strip
Assay Incubation Time	30/15 minutes	30/30/15 minutes
Reporter Conjugate	Alexa Fluor 647	Alkaline Phosphatase
Method of detection	Fluorometer	Visual

K. Standard/Guidance Document Referenced (if applicable):

FDA Guidance for the content of Premarket Submissions for Software Contained in Medical Devices.

L. Test Principle:

The ENA IgG BeadChip™ Test System is a randomly encoded microarray-based immunoassay for antibodies to extractable nuclear antigens. The device is designed to detect IgG class antibodies in human serum to six extractable nuclear antigens: SSA, SSB, Sm, RNP/Sm, Jo-1, and SCL-70. Each antigen is covalently immobilized to a spectrally distinguishable bead type. A pool of bead types is constructed by mixing all of the bead types of interest including antigen beads, positive control beads, negative control beads, and system control beads. The bead mixture is immobilized as a BeadChip™ microarray on a silicon chip allowing for the simultaneous detection of the auto-antibodies of interest by the AIS 400. Each BeadChip is identified by a carrier ID, which is bar coded on the carrier label.

Patient samples are diluted prior to incubation with the BeadChip microarray. If ENA specific antibodies are present in the sample, they will bind to the immobilized antigen on one or more bead types. After washing the unbound serum from the BeadChip, Alexa-Fluor 647 conjugated goat anti-human IgG is added to the BeadChip and briefly incubated. After removing unbound detection conjugate, the BeadChip is imaged with the Array Imaging System 400 (AIS 400) to measure the fluorescent signal associated with the conjugate bound on individual beads. The average signal intensity, coefficient of variance of the intensities, and the number of beads measured for each type is determined and reported. The ENA Analysis Software imports the instrument results, assesses the validity of the internal controls and generates test results. The ENA Analysis Software performs all calculations. The fluorescent signal is normalized based on the intensity of the conjugate control (hIgG) and characteristics of the individual serum sample. The normalized signal is compared with the predetermined analyte cut-off value and a result of positive, negative or equivocal is determined. These cutoff values are lot-specific values predetermined at BioArray Solutions. These values are stored in the CD provided in the kit and must be used with the ENA Analysis Software.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   - *a. Precision/Reproducibility:*
     - i. Study design: Reproducibility studies performed included Day-to-Day reproducibility, Operator-to-Operator reproducibility and Site-to-Site reproducibility.

       Day-to-Day reproducibility: The day-to-day reproducibility study was conducted with a pooled positive sample (PDP) containing antibodies to SSA, SSB, Sm, Sm/RNP, Jo-1, and Scl-70 and a pooled normal serum sample (PNS). The ENA IgG BeadChip assay was performed in duplicate for ten consecutive days for a total of twenty runs per sample using three lots of BeadChips. The pooled positive and negative controls were prepared, aliquoted, and stored in a -20^{o}C freezer. A new aliquot was used each day to prepare fresh dilutions. The three

lots of BeadChips were manufactured from three independently coupled ENA bead libraries.

Operator-to-Operator reproducibility: The operator-to-operator reproducibility was conducted with the pooled disease positive (PDP) control, the pooled negative control or pooled normal serum (PNS) sample and ten disease positive samples. PNS-10 is 1:10 diluted pooled normal serum sample. PDP-30 is 1:30 diluted pooled disease positive sample. Two researchers performed the ENA IgG BeadChip assays in duplicate.

Site-to-Site reproducibility: A site-to-site reproducibility study was performed to assess the assay variability between sites, instruments and operators. Identical serum samples (commercially obtained) were provided to the three testing sites for on-site testing. The three testing sites included BioArray Solutions and two clinical laboratories. These samples were randomly encoded to eliminate any references to identity or diagnosis prior to distributing the samples to the investigational sites. A total of 176 samples comprised of 52 normal samples and approximately 25 positive samples for each antigen were used. A total of 192 samples were run at each site. Ten samples were excluded due to specimen control failure at one or more sites and one sample was excluded due to a sample preparation error. Results for two samples for the Scl-70 marker were excluded due to cluster QC failure at one site. These samples were not repeated at that site.

ii. Results/Acceptance criteria: Day-to-Day Reproducibility: The results for the pooled positive sample are reported in Table 1 by antigen, including the mean of the relative activity (RA), standard deviation (SD), and the coefficient of variance (CV) for each lot (intra-lot reproducibility) and the Mean, SD, and CV of relative activity of all three lots (inter-lot reproducibility). The RA is the ratio between the antigen Normalized Intensity (NMI) and the lot specific cutoff multiplied by 100. The CVs of the RAs were less than 15%.

Table 1: Day-to-Day Reproducibility: Pooled Positive Sample

RELATIVE ACTIVITY (RA)			SSA	SSB	Sm	RNP/Sm	Jo-1	Scl-70
INTRA-LOT REPRODUC	Lot-A (n=20)	MEAN	826.1	423.4	661.1	1016.0	458.6	123.3
		SD	92.5	50.7	78.6	125.3	62.2	14.0
		CV(%)	11.2	12.0	11.9	12.3	13.6	11.3
	Lot-B (n=20)	MEAN	890.3	435.4	777.6	1198.9	549.8	135.3
		SD	76.1	64.4	123.3	120.1	69.1	18.6
		CV(%)	8.5	14.8	15.9	10.0	12.6	13.7
	Lot-C (n=20)	MEAN	776.5	442.7	751.1	1115.6	522.8	136.3
		SD	56.4	37.4	87.7	88.8	54.7	15.0
		CV(%)	7.3	8.4	11.7	8.0	10.5	11.0
INTER LOT	Lot-A,B,C (n=60)	MEAN	831.0	433.9	730.0	1110.2	510.4	131.6
		SD	88.6	51.7	109.0	133.9	72.4	16.8
		CV(%)	10.7	11.9	14.9	12.1	14.2	12.7

Day-to-Day reproducibility studies were also performed with pooled negative control samples and the results were acceptable.

Operator-to-Operator reproducibility: The %CVs were less than 10% for all positive signals; PNS-10 is a pooled negative control and as expected, the %CV for the weak nonspecific signals for each antigen are much higher when compared to disease positive markers.

Site-to-Site reproducibility: Comparative analysis of the results obtained from three sites showed that the total agreement of positive/negative calls between three sites for SSA, SSB, Sm, Sm/RNP, JO-1, and Scl-70 is 97.2%, 95.6%, 96.7%, 90.6%, 97.2% and 96.1% respectively.

Table 2: Site-to-Site reproducibility

	+/+/+	-/-/-	?/?/?	-/?	+/?	+/-	Total #	(%) Total Agreement
SSA	38	138		3	2		181	97.2
SSB	35	137	1	5	2	1	181	95.6
SM	40	135		5	1		181	96.7
RNP/Sm	38	125	1	15	1	1	181	90.6
Jo-1	37	138	1	3	1	1	181	97.2
Scl-70	27	144	1	5	2		179	96.1

Note: (+) Positive (-) Negative (?) Equivocal

b. *Linearity/assay reportable range:*
No linearity studies were performed

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Stability studies of the kit support a shelf life of 2 weeks from the date of manufacture.

d. *Detection limit:*
To determine the analytical sensitivity of the assay, the limit of blank was determined using assays performed with sample diluent in place of serum during the sample incubation. A total of seven assays were performed. The mean and SD of the RA for each antigen are given in Table 3.

Table 3: Analytical sensitivity

RA	SSA	SSB	Sm	RNP/Sm	Jo-1	Scl-70
Mean	2.4	0.3	2.0	2.9	0.4	1.2
SD	3.6	1.3	0.7	2.9	0.7	0.3
Mean +3SD	13.3	4.2	4.1	11.6	2.4	2.1

e. *Analytical specificity:*
To test whether rheumatoid factor or anti-ds DNA antibodies cross-react with the

BeadChip ENA antigen panel, two and six purchased samples characterized by the vendor as positive for rheumatoid factor and anti-ds DNA respectively were tested side-by-side with the BeadChip and the predicate device. No cross-reaction was detected from rheumatoid factor or anti-ds DNA antibodies.

Interference testing with a hemolyzed sample and a lipidemic sample showed apparent interference with the lipidemic sample, but not with the hemolytic sample. The limitations of test in package insert specify not to use any lipidemic samples. Limitation also recommends avoiding any heterophile antibodies to rabbit or bovine derived materials.

Additional tests to identify the operating range of the assay in the presence of interfering substances showed that bilirubin at concentrations of 10, 20, and 40 mg/dl and hemoglobin at concentrations of 250, 500, and 1000 mg/dl were not found to interfere with the assay within the tested limits.

f. Assay cut-off:

To determine the cut-off for each antigen in the ENA IgG BeadChip assay, 130 normal samples from healthy, asymptomatic human subjects were assayed. The mean and standard deviation of the relative activity (RA) is listed in Table 4. The upper limit of the normal range for each antigen is the mean plus 3~3.5 standard deviations (SD) and the lower limit of the positive range (Cut-off) for each antigen is the mean plus 6SD.

Table 4: Cut-off determination

(RA Value)	SSA	SSB	Sm	Sm/RNP	Jo-1	Scl-70
Data Points Used	130	130	129	130	130	130
MEAN	5.7	6.0	11.6	16.7	9.2	21.0
SD	15.9	15.5	15.0	14.0	15.1	13.2
Upper limit - normal range	60	60	60	60	60	60
Positive Cut-off	100	100	100	100	100	100
Equivocal	(60-100)	(60-100)	(60-100)	(60-100)	(60-100)	(60-100)

2. Comparison studies:

a. Method comparison with predicate device:

i. Study design: A comparative study to demonstrate the performance characteristics of the ENA IgG BeadChip Test System relative to the predicate device was conducted using 229 human serum samples from commercial vendors. The samples included 54 from normal, healthy subjects and 175 from subjects with confirmed or suspected autoimmune disease. Subject ages ranged from 19 to 77 years of age. The gender was known for 171 subjects with 39 male subjects and 132 two female subjects.

ii. Results: Samples assayed by the predicate device are positive/negative calls for each antigen. Samples assayed by BeadChips are assigned positive, negative or equivocal calls. Percent agreement between the ENA IgG BeadChip Test System and the predicate is listed in Table 5. Samples for which results could not be determined with the predicate device or were equivocal with the ENA IgG BeadChip Test System are not included in the sub-totals or the calculation of the percent agreement.

Table 5: Percent agreement between the ENA IgG BeadChip Test System and the predicate

Predicate/ BeadChip	POS/POS	NEG/NEG	POS/NEG	NEG/POS	EQU/ND	Total	% Postive Agreement	% Negative Agreement
SSA	47	159	2	10	11	229	95.9	94.1
SSB	21	195	0	8	5	229	100.0	96.1
Sm	48	156	1	10	14	229	98.0	94.0
RNP/Sm	50	155	0	14	10	229	100.0	91.7
Jo-1	21	204	0	1	3	229	100.0	99.5
Scl-70	31	183	3	2	10	229	91.2	98.9

b. Matrix comparison:
Not applicable.

3. Clinical studies:
   a. Clinical Sensitivity:
   Not applicable.
   b. Clinical specificity:
   Not applicable.
   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable.

4. Clinical cut-off:
   Same as Assay cut-off.

5. Expected values/Reference range:
   Same as Assay cut-off.

N. Instrument Name:
Array Imaging System

O. System Descriptions:
The AIS 400 system is an automated image acquisition system with an integrated analysis system designed for BioArray Solutions BeadChip™ microarrays. The major components are the microscope, automatic stage, focus motor, light source, CCD camera, computer and a software package that includes BioArray Solution Reader (BASR), Client Merger, and New Batch programs.

The BioArray Solutions Reader (BASR) is the software that controls the operation of the microscope and the acquisition of the assay images, including the auto-positioning and auto-focus features. The hardware components are responsible for acquiring the images necessary for analysis of the BeadChip microarray.

The New Batch program copies the cluster data for a new batch of BeadChips™ from the CD provided with the slides to customers hard drive. It only has to be run once for every new batch of BeadChips™.

Bioarray's Client Merger software processes the assay image and merges the assay signal with cluster information. The merge result files will be generated and saved in the cluster folder.

1. Modes of Operation:
   The AIS 400 operates in a semi-automatic fashion. The operator is required to manually position and focus on the first BeadChip on a carrier; subsequent BeadChips are positioned and focused automatically. While the instrument generally functions in a batch mode, individual BeadChips may be read selectively.

2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
   Yes ___√_____ or No _____

3. Specimen Identification:
   Specimen information is entered manually into the software program for its position on the BeadChip. Each BeadChip is identified by a carrier ID, which is bar coded on the carrier label.

4. Specimen Sampling and Handling:
   Diluted serum samples are applied directly to the top of the BeadChip by manual or automated pipetting. After incubating with diluted conjugated antibody, unbound detection conjugate is removed and the BeadChip is imaged with the AIS 400 to measure the fluorescent signal associated with the conjugate bound on individual beads.

5. Calibration:
   The system measures arbitrary fluorescent units therefore no calibration is required. However, a performance check is conducted using a company defined standard, calibration slide, to ensure the uniformity and change in intensity of illumination in the AIS 400.

6. Quality Control:
   Several controls are used in this system which includes positive and negative serum controls, a system control (internal standard), a background control (h-SA bead), a specimen control (Protein-L) and a procedural control or conjugate control (hIgG).

The system control (internal standard) is present on every BeadChip. This ensures that the AIS 400 is operating within expected parameters by monitoring the system performance and output data quality. The background control (h-SA bead) monitors non-specific binding and assay background. The specimen control (Protein L) detects the presence of immunoglobulins with kappa chains in adequate amounts. The procedural control or conjugate control (hIgG) verifies the addition of the Conjugate Detection Antibody and adequate incubation time. The positive and negative serum controls are included in each run which determines the run validity of the system. The positive serum control must be positive for each antigen, negative for the background control (h-SA bead), and positive for the specimen control. The negative serum control must be negative for each antigen, including the background control (h-SA bead), and positive for the specimen control.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

None

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.